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Phone: 202-776-0544 | Fax 202-776-0545
bloodadvances@hematology.org

CD19-positive lymphocyte count is critical for acquisition of anti-SARS-CoV-2 IgG after vaccination in B-cell lymphoma.

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Akinao Okamoto (Fujita Health University, Japan) Hidetsugu Fujigaki (Fujita Health University, Japan) Chisako Iriyama (Fujita Health University School of Medicine, Japan) Naoe Goto (Fujita Health University School of Medicine, Japan) Hideyuki Yamamoto (Fujita Health University School of Medicine, Japan) Keichiro Mihara (Fujita Health University School of Medicine, Japan) Yoko Inaguma (Fujita Health University, Japan) Yasuo Miura (Fujita Health University School of Medicine, Japan) Katsuya Furukawa (Nagoya Univ. Graduate School of Medicine,, Japan) Yukiya Yamamoto (Chubu University, Japan) Yoshiki Akatsuka (Nagoya Univ. Graduate School of Medicine,, Japan) Senji Kasahara (Gifu Municipal Hospital, Japan) Kotaro Miyao (Anjo Kosei Hospital, Japan) Masutaka Tokuda (Fujita Health University School of Medicine, Japan) Seiko Sato (Fujita Health University Hospital,, Japan) Yuki Mizutani (Fujita Health University Hospital,, Japan) Michiko Osawa (Fujita Health University Hospital,, Japan) Keiko Hattori (Fujita Health University School of Medicine, Japan) Sachiko Iba (Fujita Health University, Japan) Ryoko Kajiya (Fujita Health University School of Medicine, Japan) Masataka Okamoto (Fujita Health University Okazaki Medical Center,, Japan) Kuniaki Saito (Fujita Health University School of Medicine, Japan) Akihiro Tomita (Fujita Health University School of Medicine, Japan)

Abstract:

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CD19-positive lymphocyte count is critical for acquisition of anti-SARS-CoV-2 IgG after vaccination in B-cell lymphoma

Akinao Okamoto[1], Hidetsugu Fujigaki[2], Chisako Iriyama[1], Naoe Goto[1], Hideyuki Yamamoto[1], Keichiro Mihara[3], Yoko Inaguma[4], Yasuo Miura[5], Katsuya Furukawa[6], Yukiya Yamamoto[7], Yoshiki Akatsuka[8], Senji Kasahara[9], Kotaro Miyao[10], Masutaka Tokuda[1], Seiko Sato[11], Yuki Mizutani[11], Michiko Osawa[11], Keiko Hattori[1], Sachiko Iba[1], Ryoko Kajiya[1], Masataka Okamoto[1,12], Kuniaki Saito[2] and Akihiro Tomita[1*]

1. Department of Hematology, Fujita Health University School of Medicine, Toyoake, Japan
2. Department of Advanced Diagnostic System Development, Fujita Health University Graduate School of Health Sciences, Toyoake, Japan
3. International Center for Cell and Gene Therapy, Fujita Health University, Toyoake, Japan
4. Department of Clinical Medicine, Fujita Health University School of Medicine, Toyoake, Japan
5. Department of Blood Transfusion, Fujita Health University Hospital, Toyoake, Japan
6. Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan
7. Department of Biochemical and Sciences, Chubu University, Kasugai, Japan
8. Department of Immunology, Nagoya University Graduate School of Medicine, Nagoya, Japan
9. Department of Hematology, Gifu Municipal Hospital, Gifu, Japan
10. Department of Hematology and Oncology, Anjo Kosei Hospital, Anjo, Japan
11. Department of Clinical Laboratory, Fujita Health University Hospital, Toyoake, Japan
12. Department of Oncology, Fujita Health University Okazaki Medical Center, Okazaki, Japan

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***Corresponding Author:**

Akihiro Tomita

Department of Hematology, Fujita Health University School of Medicine

1-98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi 470-1192, Japan

Tel: +81-562-93-9243; Fax: +81-562-95-0016

E-mail: atomita@fujita-hu.ac.jp

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Letter to *Blood Advances*

SARS-CoV-2 causes more severe COVID-19 disease in patients with hematologic diseases than in healthy individuals.[1-4] Reportedly, the acquisition of antibodies after SARS-CoV-2 vaccination in these patients is also inferior to that in healthy individuals.[5-12] In particular, patients with B-cell malignancies who have been treated with anti-CD20 antibody therapeutics or Bruton's tyrosine kinase (BTK) inhibitors present much lower anti-SARS-CoV-2 antibody levels after vaccination, and the rate of antibody acquisition correlates inversely with the interval between discontinuation of the drugs and vaccination.[8,11,12] However, there is still limited information about the conditions under which such patients might acquire antibodies after vaccination. In this study, we conducted a prospective observation study (UMIN 000045150) to evaluate antibody titers achieved after SARS-CoV-2 vaccination in patients with hematological diseases, focusing on peripheral blood lymphocyte subsets and gamma globulin levels, to evaluate the response and search for markers that predict the response.

This study included patients with hematological diseases (N=263) and healthy volunteers (N=41) who were vaccinated with mRNA vaccines (BNT162b2, mRNA-1273) between May and July 2021 at Fujita Medical University and three affiliated hospitals (**Supplementary methods and Tables S1-S3**). The primary endpoint was the concentration of anti-SARS-CoV-2 receptor-binding domain (RBD) of spike protein subunit 1 conjugated IgG antibody approximately 14-28 days after administration of the second vaccine dose.[13] Secondary endpoints included the association between the acquired antibody titer and diagnosis of hematological diseases, treatment strategy, duration from the last treatment, blood cell counts, including lymphocyte subset at the time of vaccination, and serum immunoglobulin levels. Since the lowest antibody titer obtained after the second dose in healthy subjects (N=41) was 21.3 U/mL in this cohort, and all but one of the healthy volunteers in a previous study (N=219) at Fujita Health University showed an antibody titer of 20 U/mL or higher (manuscript submitted by Fujigaki *et al.*), an antibody titer of 20 U/mL or higher was used as the cut-off to define a positive antibody response after vaccination in this study. The

protocol for the experimental use of patient samples was approved by the institutional review board of Fujita Health University (HM21-067 and HM21-179). Blood samples were harvested after obtaining appropriate informed consent from all the participants. The study was conducted in accordance with the Declaration of Helsinki.

BNT162b2 was administered to 74.1% of the patient group and 100% of the healthy group. The percentage of positive results was significantly lower in the hematological disease group than the healthy group (52.5% *vs.* 100%, *p<0.001*), and antibody titers acquired after vaccination were also significantly lower (median; 23.8 *vs.* 105.6 U/mL, *p<0.001*) (**Table S1**). The results of age- and gender-matched analysis were similar (58.7% *vs.* 100%, *p<0.001*). In a comparison of patient groups with lymphoproliferative disorders (lymphoid; N=176), myeloproliferative disorders (myeloid; N=49) and benign hematological diseases (benign; N=38), antibody titers were significantly lower in the collective disease group than the healthy group (N=41), as reported previously (**Figure S2**).

We then further analyzed the lymphoid group which had a particularly low antibody acquisition rate after vaccination than other groups. In patients with lymphoid malignancies (47 with B-cell lymphoid malignancies [BCL] and four with T-cell lymphoid malignancies [TCL]) undergoing immunotherapy and/or chemotherapy (under treatment or within 3 months after the last administration of therapeutics), a positive antibody titer was not observed in any of the cases, except for one patient with TCL (**Figure 1A**, **Table S2**). Next, we examined the relationship between duration since the last administration and acquired antibody titers in BCL patients who received anti-CD20 antibody therapeutics (N=82) (**Figure 1B**). Patients within 10 months since the last dose of anti-CD20 antibody therapeutics tended to have more difficulty in acquiring antibodies than those with an interval of more than 10 months, which was consistent with previous reports.[8,11] Additionally, in our cohort, 37.8% of the patients beyond 12 months after antibody treatment (N=37) did not mount an antibody response following vaccination (**Figure 1B**). Based on these results, the factors involved in

antibody response after vaccination among patients treated with anti-CD20 antibodies were examined in detail, focusing on blood cell counts, including lymphocyte subsets, and globulin levels at the time of vaccination (**Figure S2, Table S4**). The results showed that the numbers of CD19- and CD4-positive cells in peripheral blood and serum IgM titer correlated significantly with the acquired antibody titer. These trends were also seen in patients with hematologic diseases overall, but were particularly pronounced in patients treated with anti-CD20 antibody therapeutics (**Table S5**). Using receiver operating characteristic (ROC) analyses, the cut-off values were calculated as a CD19-positive cell count of 20/μL, CD4-positive cell count of 320/μL, and serum IgM level of 20 mg/dL (**Figure S3**). With these criteria, CD19-positive cell counts in peripheral blood showed the strongest correlation with increased anti-SARS-CoV-2 IgG titers (r=0.844, *p<0.001*), with 0% antibody gain at a titer of <20/μL and 76.7% gain at >20/μL (**Figure 1C**). In addition, CD4-positive cell counts of more than 320/μL and IgM levels of more than 20 mg/dL also significantly correlated with increased antibody titers (**Figure 1D, E**), although the correlation coefficient was lower than that of CD19-positive cell count.

Finally, the same analyses were performed focusing only on cases in whom BNT162b2 was used to check if the results were biased by the type of vaccine used. Similar results were obtained for antibody acquisition after vaccination in each hematological disease, and for the relationship between peripheral blood CD19- and CD4-positive cell counts and serum IgM levels and antibody acquisition in patients treated with anti-CD20 antibody (**Figure S5**).

At present, there is no clear evidence suggesting the optimal timing of vaccination in patients with hematologic malignancies undergoing or following chemo-immunotherapy. In particular, patients after the use of anti-CD20 antibody therapeutics are less much likely to benefit from vaccination than other patients, a finding that emphasizes the need for an objective marker that can guide vaccination practice in this special population. Our analysis suggests that the number of CD19-positive cells in peripheral blood might be a useful marker to predict the likelihood of an antibody response to SARS-CoV-2 vaccination in patients with hematological diseases. Furthermore, serum IgM levels might be

useful as an alternative indicator in cases where subset analysis is difficult. One finding of this study that requires attention is that some patients who did not show low levels of CD19, CD4 or IgM also failed to acquire sufficient antibody titers following vaccination (**Figures S2 and S4**). Although lymphocyte function and/or other related mechanisms are also thought to be important for antibody acquisition, we did not analyze these functions in individual cases in this study, and this is an issue for further study.

Our findings show that the antibody response to SARS-CoV-2 vaccination is grossly suboptimal in patients with hematological disorders, especially in BCL patients undergoing treatment. Alternative preventive strategies, such as proactive vaccination of the patient's family and surrounding supporters and, in the event of close contact with an infected person, prophylactic administration of monoclonal antibodies or antivirals should be considered to protect patients receiving anti-CD20 antibody therapeutics.

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Disclosure of Conflicts of interests

K.Mihara: Research funding: TAKARA BIO. Lecture fees: Janssen Pharmaceutical, Sanofi, and CSL Behring. **Y.A.**: Honoraria: Celgene, Takeda Pharmaceutical, Kyowa Kirin, Teijin Pharma, Novartis Japan, Astra Zeneca, Sanofi, Shire Japan, Chugai Pharmaceutical, Janssen Pharmaceutical, and Bristol-Myers Squib. Advisory role: Takeda Bio. Research funding: SRL and Bristol-Myers Squib. **K.Miyao:** Lecture fees: Novartis Japan, Kyowa Kirin, Nippon Shinyaku, Takeda Pharmaceutical, Eisai, Janssen Pharmaceutical, Bristol-Myers Squib, Celgene, Ono Pharmaceutical, and Otsuka Pharmaceutical. **K.S.**: belongs to an endowed department sponsored by FUJIFILIM Wako Pure Chemical Corporation. **A.T.**: Research funding: Chugai Pharmaceutical, Astellas Pharma, Eisai, Otsuka Pharmaceutical, Ono Pharmaceutical, Kyowa Kirin, Shionogi, Sumitomo Dainippon Pharma, Taiho Pharmaceutical, Takeda Pharmaceutical, Teijin, Nippon Shinyaku, Nihon Pharmaceutical, Pfizer Japan, Mochida Pharmaceutical, Yakult Honsha, and Perseus Proteomics. Lecture fees: Chugai Pharmaceutical, Kyowa Kirin, Eisai, Takeda Pharmaceutical, Astellas Pharma, Nippon Shinyaku, Janssen Pharmaceutical, Zenyaku Kogyo, AbbVie GK, Bristol-Myers Squibb, and SymBio Pharmaceutical.

Author contributions

A.O., A.T., H.F., K.S., C.I., N.G., H.Y. and M.O. designed the study; A.T., A.O., C.I., N.G., H.Y., M.O., K.Mihara, Y.I., Y.Miura, K.F., Y.Y., Y.A., S.K., K.Miyao and M.T. collected patient blood samples; A.T., A.O., C.I., N.G., H.Y., M.O., K.Mihara, Y.I., Y.Miura, K.F., Y.Y., Y.A., S.K., K.Miyao, K.H., S.I. and Y.K. collected clinical data; H.F. and K.S. performed measurement of anti-SARS-CoV-2 antibody titers; S.S., Y.Mizutani and M.O. performed

lymphocyte subset analysis; A.O. performed statistical analysis; A.O. and A.T. generated figures and tables; A.O., A.T., C.I. and N.G. wrote the paper; all the authors participated in discussions and interpretation of the data and results.

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Figure legend

Figure 1 Acquisition of anti-SARS-CoV-2 IgG after vaccination in patients with malignant lymphoma

(A) Anti-SARS-CoV-2 IgG titers increased after the first and second vaccine doses in healthy volunteers (blue dots) and patients under treatment for malignant lymphoma (red dots). Patients under treatment were defined as those on a treatment regimen or within 3 months after the last dose of medication. **(B)** Differences in antibody titers after the second dose in patients with B-cell malignancies who were under treatment regimens including anti-CD20 antibody therapeutics. IgG titers after the second dose in the same group with appropriate laboratory data were stratified according to the respective cut-off values of pre-vaccination peripheral blood CD19-positive cell count (20 cells/μL) **(C)**, peripheral blood CD4-positive cell count (320 cells/μL) **(D)**, and serum IgM level (20 mg/dL) **(E)**, respectively. The cut-off values were calculated by analysis of the receiver operating characteristic (ROC) curve shown in **Figure S2.**
Pre; before vaccination, adm; administration, Treatment start-3M; Patients under therapy or up to 3 months after completion of the treatment regimen.

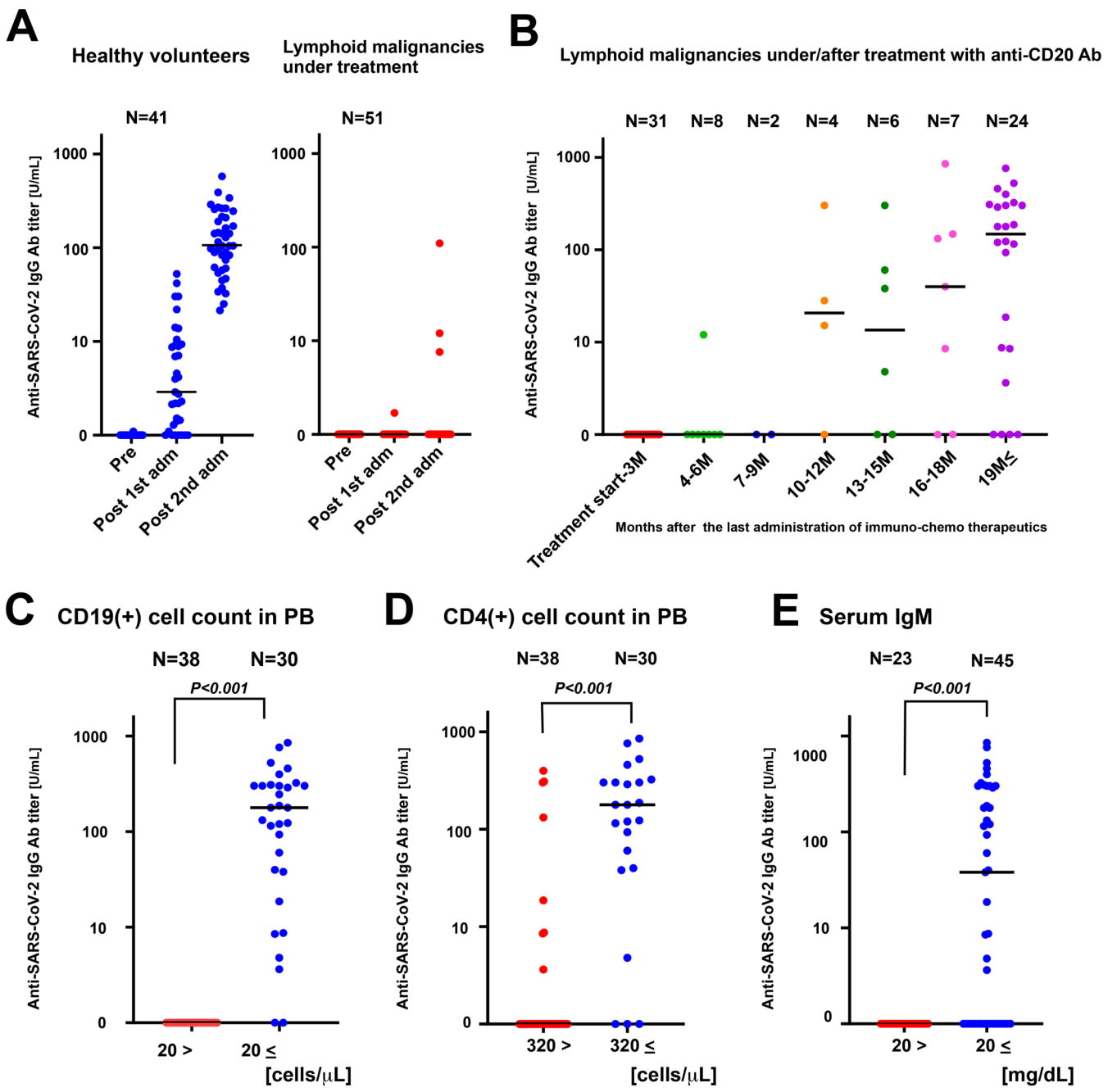
A
Healthy volunteers
Lymphoid malignancies under treatment
N=41
N=51
Anti-SARS-CoV-2 IgG Ab titer [U/mL]
1000
100
10
0
Pre
Post 1st adm
Post 2nd adm
B
Lymphoid malignancies under/after treatment with anti-CD20 Ab
N=31
N=8
N=2
N=4
N=6
N=7
N=24
Treatment start-3M
4-6M
7-9M
10-12M
13-15M
16-18M
19M≤
Months after the last administration of immuno-chemo therapeutics
C
CD19(+) cell count in PB
N=38
N=30
P<0.001
20 >
20 ≤
[cells/μL]
D
CD4(+) cell count in PB
320 >
320 ≤
E
Serum IgM
N=23
N=45
[mg/dL]

Figure 1